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# Multiple approaches to assess fourteen non-invasive serum indexes for the diagnosis of liver fibrosis in chronic hepatitis C patients

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ABSTRACT

**Background:** The aim of this study was to compare fourteen non-invasive indexes/scores: AAR, APRI, Fibroindex, MODEL3, Forns index, FIB4, GUCI, FI, FCI, Pohl score, AP index, CDS, HGM-1 and HGM-2, in order to diagnose the hepatic fibrosis stage in a survey of patients with chronic hepatitis C.

**Methods:** 84 patients with chronic hepatitis C were studied. Liver fibrosis was staged according to the Scheuer scoring system. The diagnostic accuracy of these indexes/scores was evaluated by AUROC, contingency tables and logistic regression analysis.

**Results:** The best AUROCs (>0.9) to discriminate cirrhosis (F = 4), were observed for CDS, FI, AAR, MODEL3, FIB4, HGM-2 and FCI. To discriminate *at least advance fibrosis* (F ≥ 3), the best AUROCs (>0.89) were for CDS, FI, FIB4, HGM2-2, MODEL3 and FCI. To discriminate *at least significant fibrosis* (F ≥ 2), the best AUROCs (>0.8) were for FIB4, GUCI, APRI, FI, Forns index, HGM-2 and FCI. Contingency tables and logistic regression analysis supported the results obtained by AUROC.

**Conclusions:** This study compares the diagnostic performance of fourteen indexes for the diagnosis of liver fibrosis stage in the same group of CHC patients. These results allow the selection of the best indexes for further studies in larger populations, in order to build diagnostic algorithms as an alternative to liver biopsy for fibrosis staging in patients with chronic HCV infection. These algorithms would allow to take therapeutical decisions and the continuous follow-up of hepatic fibrosis in these patients.



## 1. Introduction

Worldwide, hepatitis C virus (HCV) is one of the major causes of chronic liver diseases. Hepatic manifestations of this disease include inflammation, fibrosis and cirrhosis [1]. Precise staging of liver fibrosis is essential in the management and follow-up of this liver disease. Although liver biopsy is still considered as the gold standard for grading necroinflammatory activity and staging fibrosis, it presents some technical limitations and risks (sampling error, intra- and inter-observer variation, iatrogenic injuries) in addition to its high cost [2,3]. Usually biopsy is not well accepted by patients, and clinicians cannot repeat it too often. Taking into account these limitations, nowadays it is essential to develop non-invasive methods for the diagnosis of hepatic fibrosis stage in order to decide the initiation of antiviral treatments, to evaluate

*Abbreviations:* HCV, hepatitis C virus; AAR, aspartate alanine aminotransferase ratio; APRI, aspartate aminotransferase to platelet ratio index; GUCI, Goteborg University Cirrhosis Index score; FI, fibrosis index; FCI, fibrosis cirrhosis index; CDS, cirrhosis discriminant score; HGM-1 and HGM-2, Hospital Gregorio Marañón; CHC, chronic hepatitis C; MDR-4, Modification of Diet in Renal Disease study; AST, aspartate aminotransferase; ALT, alanine aminotransferase; UPN, upper limit of normality; PTL, platelets; Ig, immunoglobulin; TP, total protein; INR, international normalized ratio; GGT, gamma glutamyl transpeptidase; CHOL, cholesterol; Alb, albumin; ALP, Alkaline phosphatase; Bb, bilirubin; GLU, glucose; CI, confidence interval; ROC, receiver operating characteristic curve; AUROC, area under the receiver operating characteristic curve; Se, sensitivity; Sp, specificity; PPV, positive predictive value; NPV, negative predictive value; DOR, diagnostic odds ratio; J, Youden's index; LR, likelihood ratio; LRA, logistic regression analysis; DANA, difference between Advanced and No Advanced fibrosis.

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progression/regression of the hepatic disease, and to assess the effectiveness of treatments. Fibroscan (transient elastography) is a recently validated non-invasive technique but it is far from being considered a perfect gold standard, it presents technical and economical limitations (patients with a narrow inter-costal space, the presence of ascites, high body mass index of patients, presence of visceral adiposity, cost of the fibroscan device, need of specialized technicians). Considering these limitations, many studies have been recently focused on the development of non-invasive markers as surrogates for liver biopsy. [4–7].

The aim of the present study is to compare the diagnostic performance of 14 non-invasive, non-patented indexes and scores: AAR, APRI, Fibroindex, MODEL3, Forns index, FIB4, GUCI, FI, FCI, Pohl score, AP index, CDS, HGM-1 and HGM-2, for the diagnosis of hepatic fibrosis stage in a survey of patients with chronic hepatitis C, in order to: 1) build in a near future diagnostic algorithms, or decision trees, as an alternative to liver biopsy for fibrosis staging in patients with chronic HCV infection, and 2) get a regular follow-up of the evolution of these patients.

This study compares in the same survey, by ROC curves (AUROCs), contingency tables and logistic regression analysis (LRA), the diagnostic performance of these not patented indexes/scores for the diagnosis of liver fibrosis stage. This preliminary study allow us to choose the best indexes for further studies in larger populations, in order to build diagnostic algorithms as an alternative to liver biopsy for fibrosis staging in patients with chronic HCV infection. These algorithms would permit to take therapeutical decisions and the continuous follow-up of the hepatic fibrosis in these patients.

Finally, some of the evaluated indexes had been designed for co-infected patients (HIV–HCV) but, in the present study, they have been used to assess mono infected patients (HCV) obtaining good results.

## 2. Materials and methods

### 2.1. Patients

This survey included 84 consecutive prospective adult patients with chronic hepatitis C (CHC) who were scheduled to have a percutaneous liver biopsy from January 2008 to January 2012 at the University Hospital "Lozano Blesa" of Zaragoza (Spain). Patients were informed of the objectives of the study, written informed consent was obtained and fasting blood samples were collected the same day immediately before liver biopsy. All parameters were routinely determined in the clinical laboratory. The medical history of the patients was reviewed. Demographic, laboratory and clinical variables were recorded.

The diagnosis of CHC was established by the presence of HCV RNA using polymerase chain reaction assays. Patients with the following conditions were excluded from the study: alcohol consumption (>20 g/day for females, >30 g/day for males); kidney insufficiency — defined as Modified Diet in Renal Disease study equation (MDRD-4) <60 —, MDRD-4 = 186 × (Creatinine mg/dL)$^{-1.154}$ × (age)$^{-0.203}$ × 0.742 (if Female) × 1.212 (if Black) [8]; presence of other causes of liver disease; clinical evidence of decompensated cirrhosis; hepatocellular carcinoma; prior liver transplantation; prior interferon therapy; immunosuppressive therapy; and biopsies of poor quality (insufficient liver sample for fibrosis staging, <15.00 mm, or fragmented biopsy). The present study was approved by the local ethics committee (CEICA).

### 2.2. Indexes/scores

Formulas for calculating ratios, scores and indexes were taken from original publications:

- AAR = AST [IU/L]/ALT [IU/L] [9,10]
- APRI = (AST[IU/L]/ULN)/PTL[$10^9$/L] ULN Male 40 [IU/L]; ULN Female 32 [IU/L] [11]
- Fibroindex = 1.738–0.064(PTL[$10^4$/mm$^3$]) + 0.005(AST [IU/L]) + 0.463(gamma globulin [g/dL])
  Gamma globulin [g/dL] = (area gamma% x TP [g/dL]) [12]
- MODEL 3 = exp.(logodds)/(1 + exp.(logodds))
  Logodds = −5.56 − 0.0089 × PTL[$10^9$/L] + 1.26 × AST/ALT + 5.27 × INR [13]
- Forns index = 7.811 − 3.131 × ln(PTL[$10^9$/L]) + 0.781 × ln(GGT [IU/L]) + 3.467 × ln age − 0.014 × CHOL[mg/dL] [14]
- FIB 4 = age × AST [UI/L]/(PTL [$10^9$/L] × (ALT [UI/L])$^{1/2}$) [15]
- GUCI = (AST[IU/L]/ULN) × INR × 100)/PTL[$10^9$/L] [16]
- FI = 8 − 0.01 × PTL[$10^9$/L] − Alb [g/dL] [17]
- FCI = ALP[IU/L] × Bb [mg/dL]/(Alb [g/dL] × PTL[$10^9$/L]) [18]
- HGM-1 = 1/(1 + e (1.97 + 0.012 × PTL [$10^9$/L] − 0.026 × AST [IU/L] − 0.033 × GLU [mg/dL])) [19]
- HGM-2 = 1/(1 + e (6.175 − 0.010 × ALP [IU/L] − 4.8 × INR + 0.010 × PTL [$10^9$/L] − 0.007 × AST [IU/L])) [19]
- Pohl score (positive/negative) AAR = AST/ALT
  Positive: if AAR ≥ 1 and PTL < 150 × $10^9$/L [20]
- AP index (0–10) = Age (value) + PTL(value)
  Age <30 = 0, 30–39 = 1; 40–49 = 2; 50–59 = 3; 60–69 = 4; ≥70 = 5
  PTL: ≥225 = 0, 200–224 = 1; 175–199 = 2; 150–174 = 3; 125–149 = 4; <125 = 5 [21]
- CDS score (0–11) = PTL(value) + ALT/AST (value) + INR (value)
  PTL: >340 = 0; 280–339 = 1; 220–279 = 2; 160–219 = 3; 100–159 = 4; 40–99 = 5; <40 = 6
  ALT/AST: >1.7 = 0, 1.2–1.7 = 1, 0.6–1.19 = 2, <0.6 = 3
  INR: <1.1 = 0; 1.1–1.4 = 1; >1.4 = 2 [22]

### 2.3. Liver biopsy

Liver fibrosis was evaluated according to Scheuer scoring system (F0 to F4 including intermediate stages). All liver biopsies were reviewed by the same pathologist, who had no knowledge of the clinical characteristics of the study subjects. Histological results were used as reference for the evaluation of noninvasive indexes/scores. *At least significant fibrosis* was defined as Scheuer score of 2 or more (F ≥ 2), *at least advanced fibrosis* as Scheuer score of 3 or more (F ≥ 3) and cirrhosis as Scheuer score of 4 (F = 4).

### 2.4. Statistical analysis

Absolute frequencies, percentages and 95% confidence intervals (95% CI) were used to describe qualitative variables. Quantitative variables were described using range, mean and standard deviation (SD). The normality of these variables was checked with the Kolmogorov–Smirnov test. Receiver operating characteristic curves (ROC) were plotted and the diagnostic value of each index was assessed by the area under the receiver operating characteristic curves (AUROCs). Maximum Youden index (Se + Sp − 100) or the minimum euclidean distance (square root of (100 − Se)$^2$ + (100 − Sp)$^2$) [23] were used to select the optimum cutoff value for the studied diagnostic scores and indexes. Sensitivity (Se), specificity (Sp), predictive values (PPV, NPV) and accuracy were calculated using WinEpi (http://www.winepi.net). In order to check the significance of these values, Pearson's Chi square ($\chi^2$) test was used, and the Fisher's exact test was used instead when appropriate. The diagnostic odds ratio (DOR) is defined as the quotient of the positive likelihood ratio (LR+) and the negative likelihood ratio (LR−). Logistic regression analysis (LRA) was used to generate predictive models; logarithmic transformation of some indexes was calculated to normalize the values (AAR, APRI, FIB4, GUCI and FCI); step-forward method was used to include significant variables, and predictive performance of the model was assessed with Nagelkerke's $R^2$. Statistical analysis was performed by IBM SPSS Statistics 19.0 (SPSS Inc., Chicago, IL). Alpha error was established at 0.05, so p-values less than 0.05 were considered statistically significant.